SUPPLEMENTAL FIGURES

Sammels et al. (2010) – "Polycystin-2 activation by inositol 1,4,5-trisphosphate-induced Ca^{2+} release requires its direct association with the inositol 1,4,5-trisphosphate receptor in a signaling microdomain"

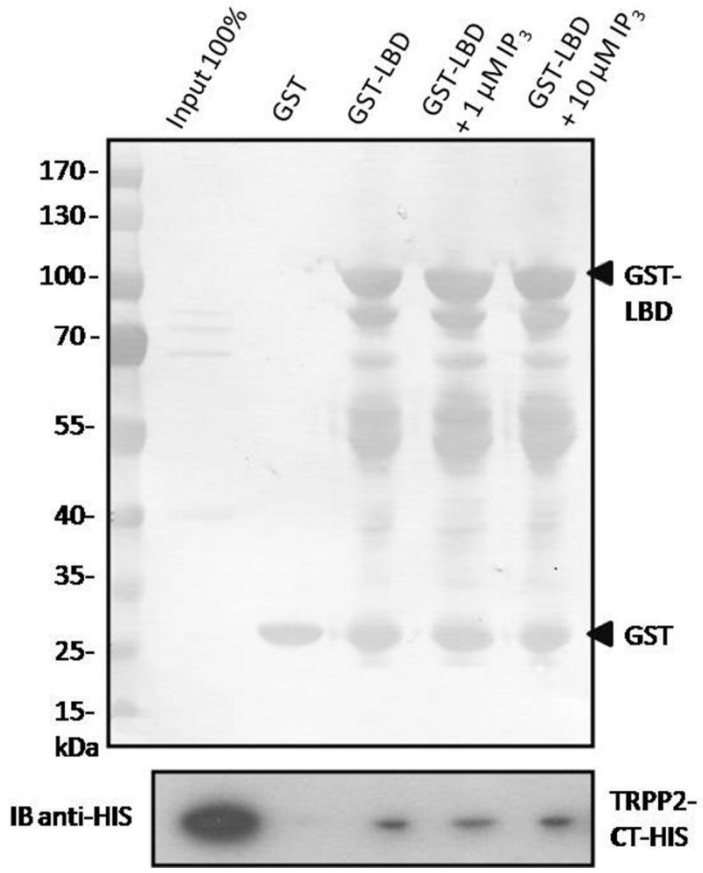

FIGURE S1. **No effect of IP_3 on the interaction between TRPP2 and the IP_3R.** GST-pull down using GST or GST-LBD and TRPP2-CT-HIS in the presence of 0, 1 or 10 μM IP_3. The upper panel shows a Ponceau-red staining of the input GST-fusion proteins. The lower immunoblot shows the staining for the HIS-fusion protein TRPP2-CT-HIS.

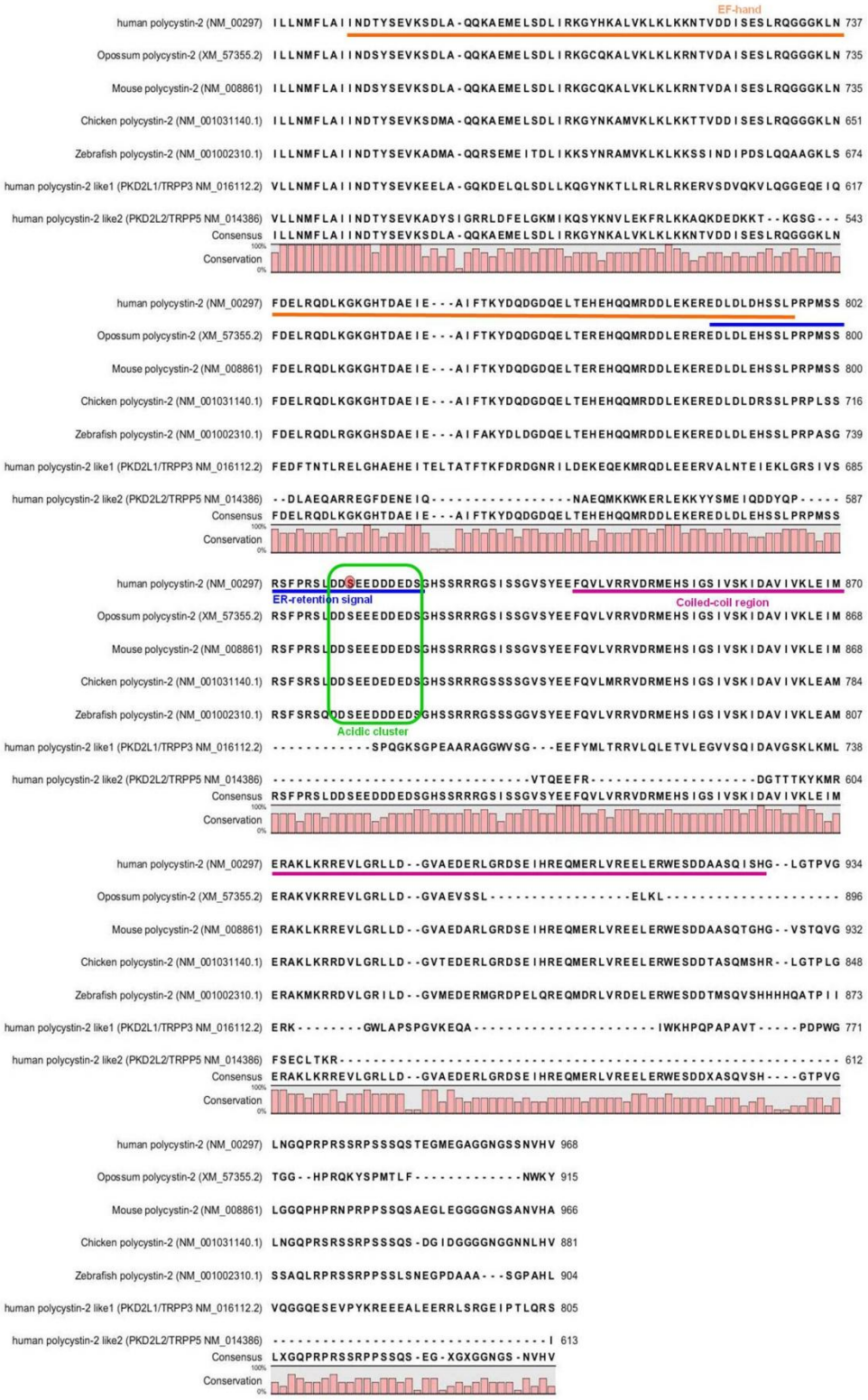
EF-hand
human polycystin-2 (NM_00297) 737
Opossum polycystin-2 (XM_57355.2) 735
Mouse polycystin-2 (NM_008861) 735
Chicken polycystin-2 (NM_001031140.1) 651
Zebrafish polycystin-2 (NM_001002310.1) 674
human polycystin-2 like1 (PKD2L1/TRPP3 NM_016112.2) 617
human polycystin-2 like2 (PKD2L2/TRPP5 NM_014386) 543
Consensus
Conservation
100%
0%
human polycystin-2 (NM_00297) 802
Opossum polycystin-2 (XM_57355.2) 800
Mouse polycystin-2 (NM_008861) 800
Chicken polycystin-2 (NM_001031140.1) 716
Zebrafish polycystin-2 (NM_001002310.1) 739
human polycystin-2 like1 (PKD2L1/TRPP3 NM_016112.2) 685
human polycystin-2 like2 (PKD2L2/TRPP5 NM_014386) 587
Consensus
Conservation
100%
0%
human polycystin-2 (NM_00297) 870
ER-retention signal
Coiled-coil region
Opossum polycystin-2 (XM_57355.2) 868
Mouse polycystin-2 (NM_008861) 868
Chicken polycystin-2 (NM_001031140.1) 784
Zebrafish polycystin-2 (NM_001002310.1) 807
Acidic cluster
human polycystin-2 like1 (PKD2L1/TRPP3 NM_016112.2) 738
human polycystin-2 like2 (PKD2L2/TRPP5 NM_014386) 604
Consensus
Conservation
100%
0%
human polycystin-2 (NM_00297) 934
Opossum polycystin-2 (XM_57355.2) 896
Mouse polycystin-2 (NM_008861) 932
Chicken polycystin-2 (NM_001031140.1) 848
Zebrafish polycystin-2 (NM_001002310.1) 873
human polycystin-2 like1 (PKD2L1/TRPP3 NM_016112.2) 771
human polycystin-2 like2 (PKD2L2/TRPP5 NM_014386) 612
Consensus
Conservation
100%
0%
human polycystin-2 (NM_00297) 968
Opossum polycystin-2 (XM_57355.2) 915
Mouse polycystin-2 (NM_008861) 966
Chicken polycystin-2 (NM_001031140.1) 881
Zebrafish polycystin-2 (NM_001002310.1) 904
human polycystin-2 like1 (PKD2L1/TRPP3 NM_016112.2) 805
human polycystin-2 like2 (PKD2L2/TRPP5 NM_014386) 613
Consensus
Conservation
100%
0%

FIGURE S2. **Sequence of the C-terminus of TRPP2.** Alignment of the sequence of the C-terminus of TRPP2 in human, mouse, rat, chicken, zebrafish and polycystin-2 like1 (PKD2L1 or TRPP3) or -2 (PKD2L2 or TRPP5) is shown (made in CLC Sequence Viewer 6). Several domains are indicated on the human TRPP2 sequence: EF-hand (1,2) (orange), the ER-retention signal (3) (blue), the acidic cluster (green), S^{812}, a CK2 phosphorylation site (4) (encircled) and the coiled-coil region (2,5) (purple).

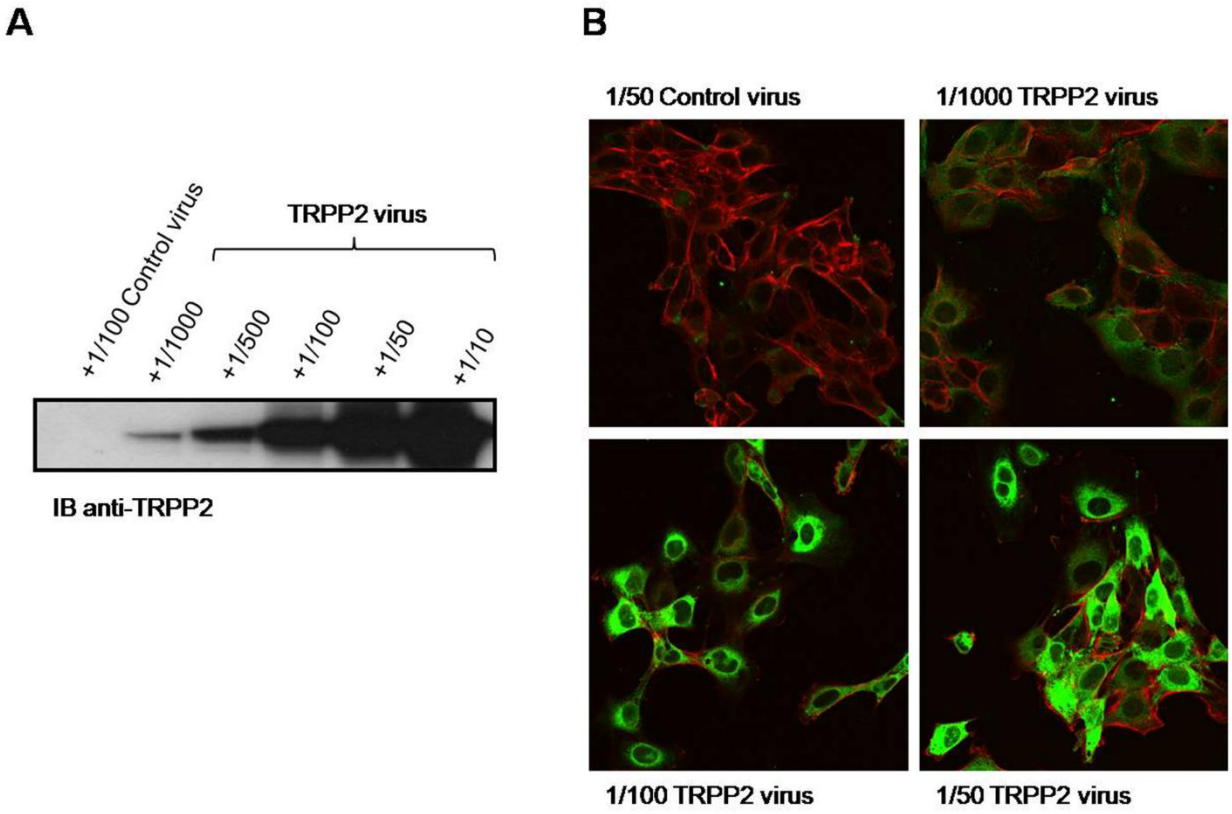

FIGURE S3. **Adenoviral expression of wild-type TRPP2.** TRPP2$^{-/-}$ cells were transduced with the indicated titers of an adenovirus for wild-type TRPP2. *A*, an immunoblot with staining for TRPP2. *B*, confocal pictures of the cells with a red staining for F-actin and a green staining for the myc-tagged TRPP2. All images were made confocally with the same settings.

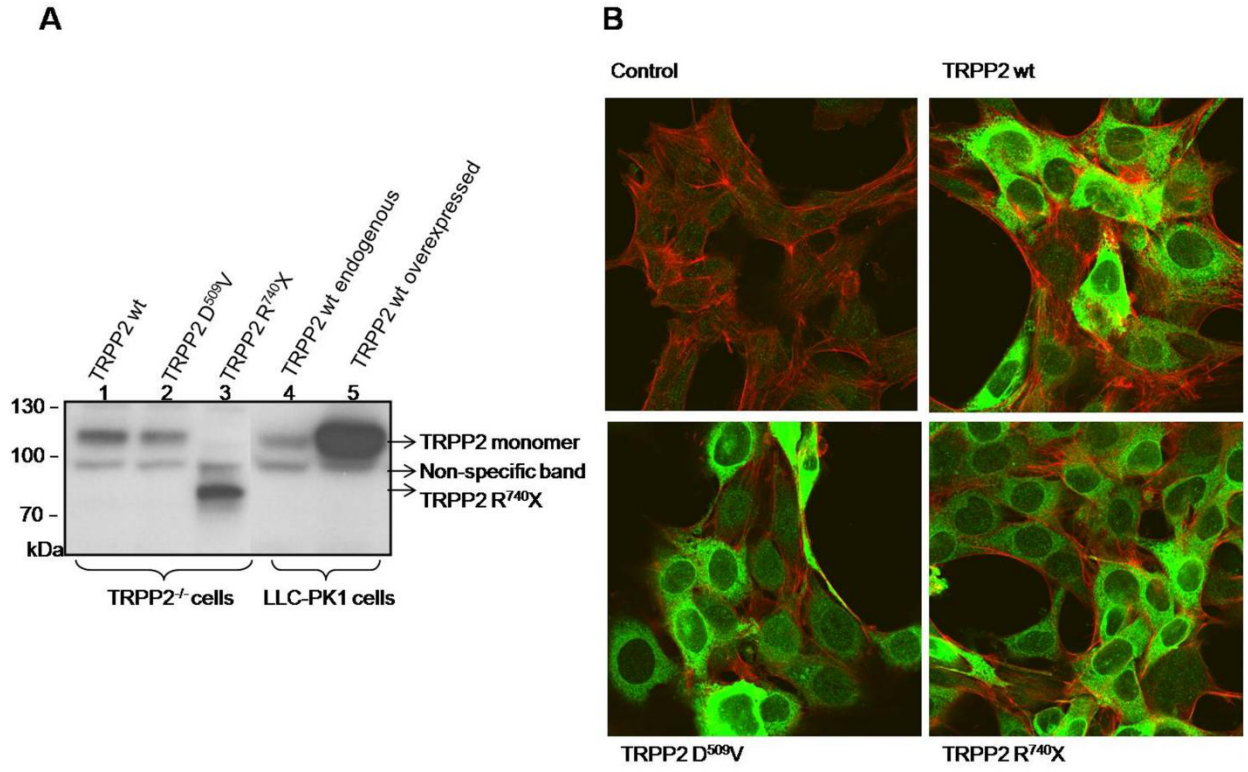

FIGURE S4. **Adenoviral expression of TRPP2 mutants.** TRPP2$^{-/-}$ cells were transduced with a control adenovirus or a TRPP2 adenovirus to re-introduce wild-type TRPP2 or the mutants $D^{509}V$ and $R^{740}X$. *A,* 15 µg lysate of transduced TRPP2$^{-/-}$ cells (lane 1-2-3) or LLC-PK1 cells endogenously expressing TRPP2 (lane 4) or over-expressing TRPP2 (obtained by plasmid over-expression) (lane 5) was analysed by SDS-PAGE. An immunoblot is shown with staining for TRPP2 using an antibody directed against the common N-terminal sequence of TRPP2. *B,* confocal pictures of the TRPP2$^{-/-}$ cells with a red staining for F-actin and a green staining for TRPP2. All images were made confocally and with the same settings. Background signal in cells treated with a control virus is shown in the upper left panel.

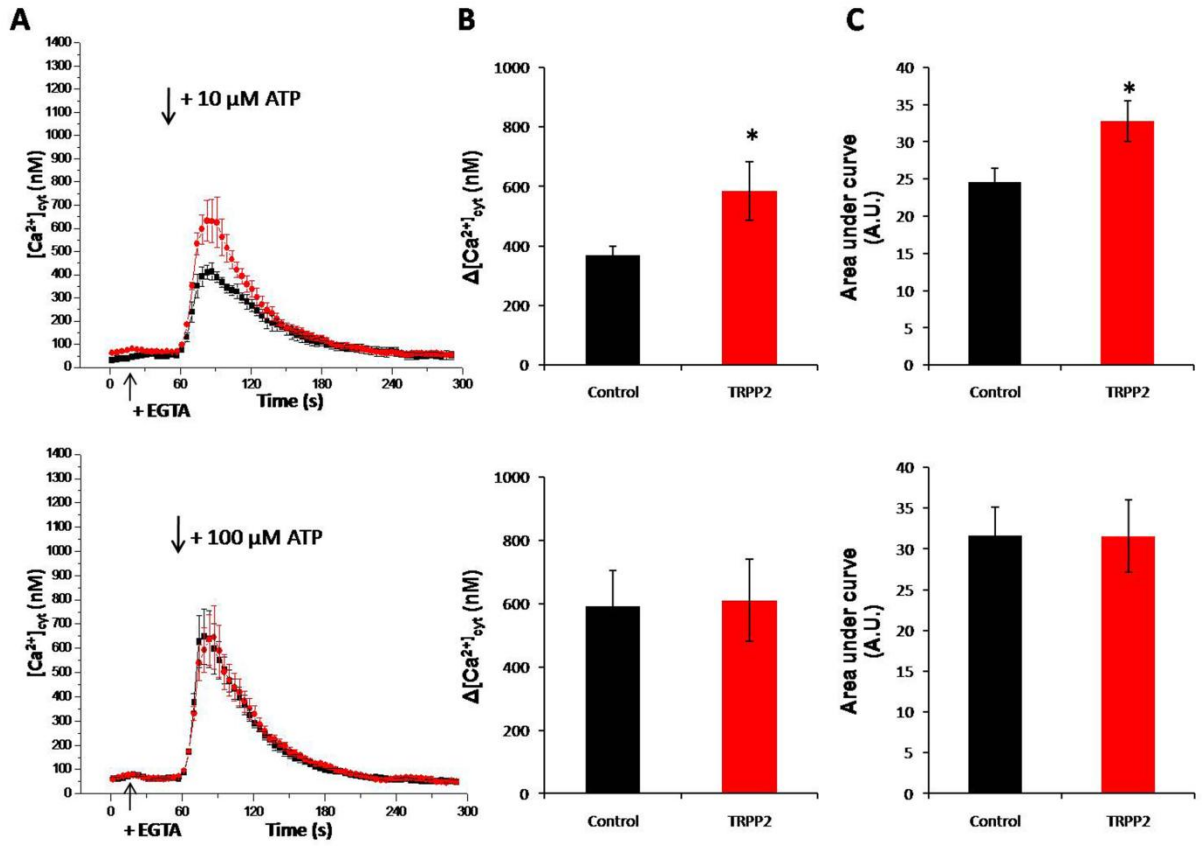

FIGURE S5. **Effect of TRPP2 on a submaximal and maximal agonist-induced Ca^{2+} release in intact cells.** $TRPP2^{-/-}$ cells were treated with either control virus (black) (titer 1/1000) or a TRPP2 virus to re-introduce wild-type TRPP2 (red) (titer 1/1000). The ratio of emitted fluorescence of Fura2 was monitored and 10 and 100 µM ATP were added as indicated in the presence of 3 mM EGTA. $[Ca^{2+}]_{cyt}$ was derived after *in situ* calibration. Panel *A* shows the averaged traces of at least three independent experiments. Panel *B* shows the increase in $[Ca^{2+}]_{cyt}$ (nM) after agonist addition, averaged for at least three independent experiments. Panel *C* shows the quantification of the area under the curve in A.U. Results are shown as means ± S.E.M.. Statistically significant differences are labelled with * for $p<0.05$, using a Student t-test (paired two-tailed).

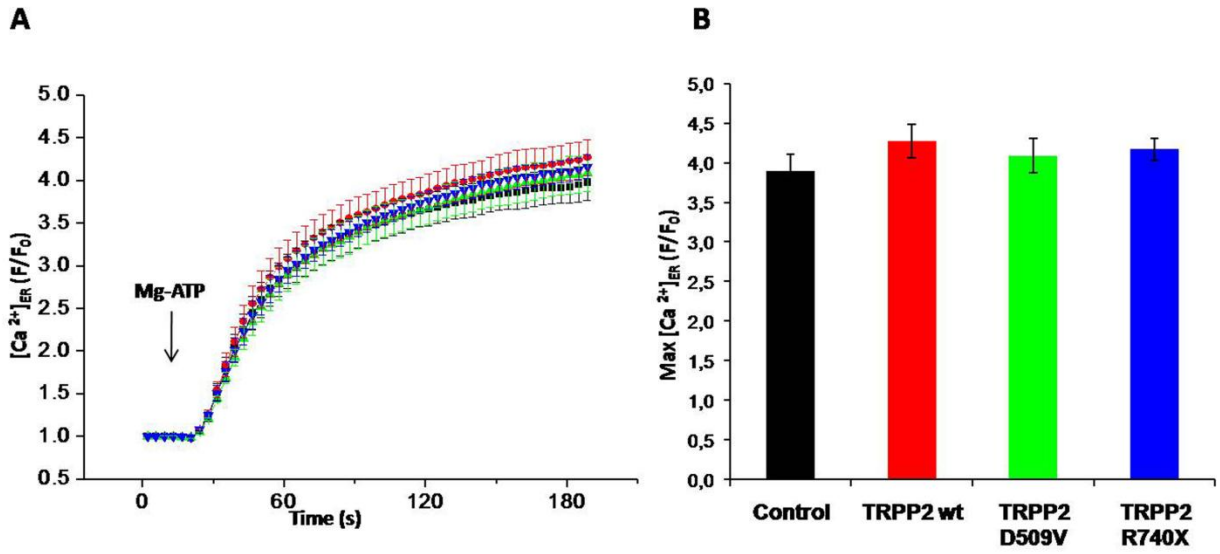

FIGURE S6. **ER loading in permeabilized cells transduced with different adenovirus preparations.** *A*, Ca^{2+} uptake by the ER in $TRPP2^{-/-}$ cells transduced with a control virus (black) (titer 1/1000) or a TRPP2 virus to re-introduce wild-type TRPP2 (red) (titer 1/1000) or the mutants $D^{509}V$ (green) (titer 1/704) and $R^{740}X$ (green) (titer 1/1124). Ca^{2+} uptake in permeabilized cells was initiated by ATP addition. Results are expressed as F/F_0 of MagFluo4 fluorescence (means ± S.E.M. for three independent experiments). *B*, maximal F/F_0 ± S.E.M. for the four conditions.

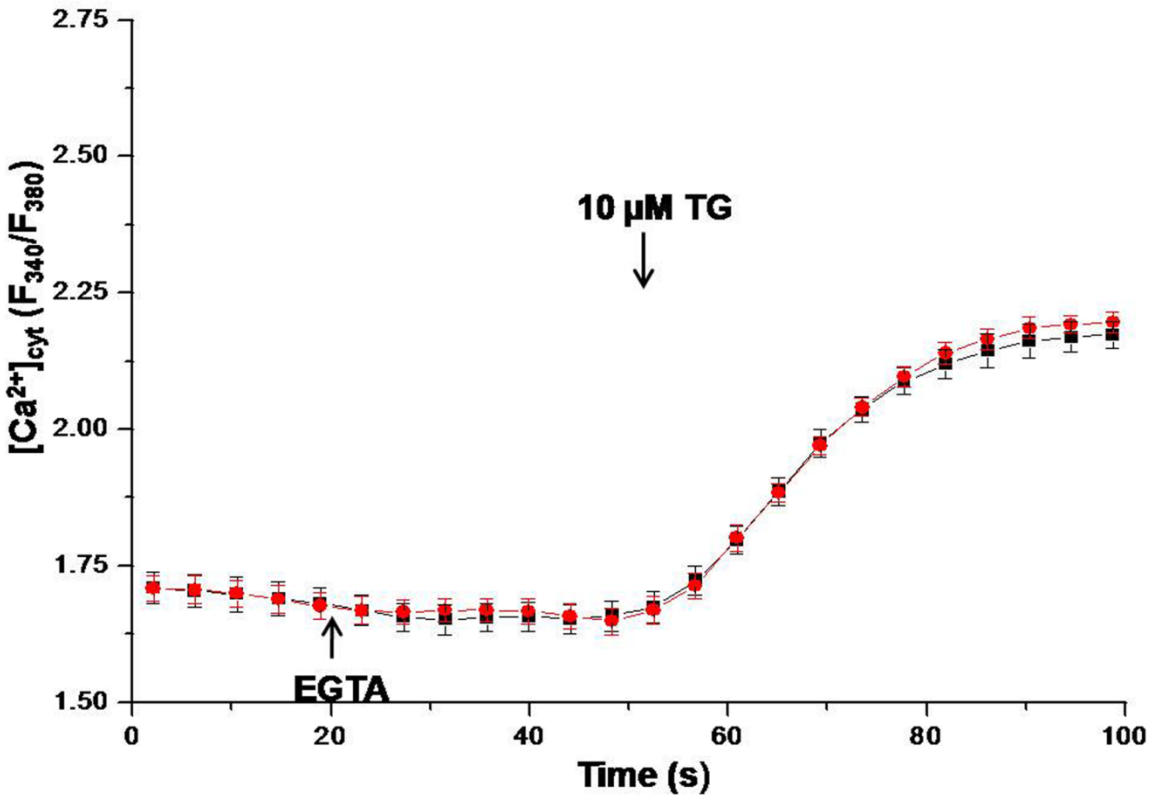

FIGURE S7. **Effect of TRPP2 on TG-induced Ca^{2+} release in intact cells.** $TRPP2^{-/-}$ cells were treated with either a control virus (black) (titer 1/1000) or a TRPP2 virus (red) (titer 1/1000) to re-introduce wild-type TRPP2. The ratio of emitted fluorescence of Fura2 was monitored and 10 µM TG was added as indicated in the presence of 3 mM EGTA. Results are shown as mean $[Ca^{2+}]_{cyt}$ (as F_{340}/F_{380}) ± S.E.M. for four independent experiments.

REFERENCES

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